J. Adelaide Bot. Gard. 1(1) 37-54 (1976)

MORPHOLOGICAL VARIATION AND TAXONOMY OF *ISOETES MUELLERI* A. BR.

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Abstract

Characters used in *Isoetes* L. taxonomy are examined for the *I. muelleri* A. Br. complex. The characters examined in detail include general morphology (based on field studies as well as dried specimens), stomata, megaspore form, size and ornamentation, sporangial characteristics and cytology.

Three classes of megaspore types are defined for species of *Isoetes* producing polymorphic megaspores.

A polyploid series in *I. muelleri* was noted with somatic chromosome numbers of 22, 44, and 55 recorded. This species is considered to be apomictic.

These studies indicate that *I. muelleri* is an exceptionally variable species occurring in a wide range of habitats throughout Australia. *I. stuartii* A. Br. is shown to be synonomous with *I. muelleri.*

Introduction

Isoetes muelleri was described by Alexander Braun in 1868 on the basis of a collection from near Rockhampton, Queensland. This species remained almost unknown, except for the original description, until Aston (1973) recorded it from the Northern Territory, South Australia, Victoria, Western Australia and Queensland. However, Aston did not discuss this or other Australian species in detail, and since Braun (1868) there has been no critical review of the Australian taxa of *Isoetes.*

I. muelleri belongs to a small group of Australian species, which also includes *I. humilior* and *I. stuartii,* characterized by the presence of vela covering the sporangia. Within this group *I. humilior* F. Muell. ex A.Br. (=*I. hookeri* A. Br.) and *I. stuartii* A. Br. differ from *I. muelleri* in only a few features (Braun, 1868; Pfeiffer, 1922) and their taxonomic status is reviewed. In this paper, characters used in *Isoetes* taxonomy are examined for the *I. muelleri* complex.

Materials and Methods

Both fresh and dried materials were examined. Collections and voucher specimens made during this study are lodged at AD. Plants were grown either submerged in a large glass tank or in a wet house with daily mist watering.

Megaspores were examined by light and scanning electron microscopy. Megaspore diameter measurements were made using dry spores, loose on microscope slides. Spores for scanning electron microscopy were fixed to small circular glass coverslips with a synthetic rubber adhesive, placed in an enclosed glass chamber and exposed to the fumes of 2% osmic acid solution overnight. This pretreatment helped reduce charging of specimens during examination (Pfefferkorn, 1970). The coverslips were then glued onto S.E.M. stubs and coated with pure gold in either an evaporative or sputter coating unit.

Specimens were examined and photographed using an ETEC Autoscan fitted with an NEC secondary X-ray detector and analyser.

Large root-tips from short, young, unbranched roots were used for chromosome preparations. The root-tips were pretreated with 20 ppm chloro-I.P.C. for 4 hours at

room temperature. This caused chromosome contraction in the same way as described for I.P.C. (Storey and Mann, 1967). Colchicine was found to be ineffective on the *Isoetes* species studied.

The pretreated root-tips were fixed in 3:1 absolute ethanol: glacial acetic acid for 20 minutes, and transferred to a mixture of approximately 0.2% cellulase and 0.5% pectinase in phosphate buffer at pH 5.2, and left overnight to soften the cell walls and intercellular pectins. This facilitated squashing of the root-tip cells. After a brief wash in 45% acetic acid, squash preparations were made from the root-tips in lacto-propionic orcein (Dyer, 1963). This procedure yielded well stained chromosomes with less cytoplasmic and background staining than with aceto-orcein or aceto carmine stains.

General Morphology

I. muelleri is variable in form (fig. la-g), ranging from tall, erect, flaccid, aquatic plants (fig. la) to small amphibious plants (fig. lg) with spreading, usually turgid leaves. Between these extremes a wide range of intermediates can be found, including tiny grass-like plants (fig. lf) which grow in dense clumps. All plants shown in figures la-f bore sporangia containing mature megaspores.

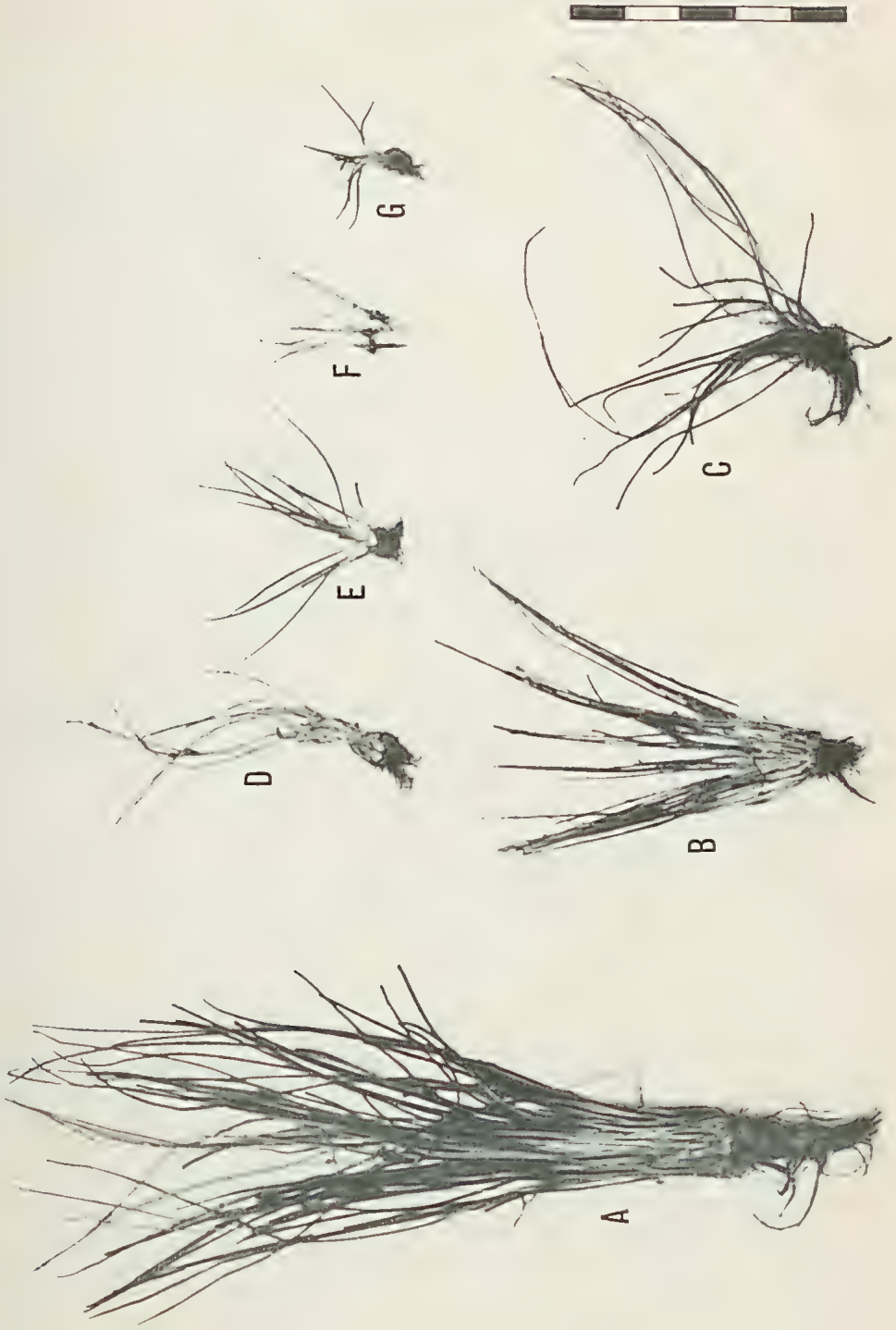
The size of plants, however, varies within individual populations. Figure 2a-d shows a range of plants collected from within a few centimetres of each other. Each plant bore mature sporangia, and most of the variation in size between them appeared to be due to age differences rather than environmental effects since all plants were found growing together in the centre of a shallow swamp.

Culturing of plants has shown that leaf habit varies under differing growth conditions. Terrestrial plants normally have only spreading leaves, whilst aquatic plants mostly have erect, flaccid leaves. When plants with spreading leaves were grown in water, new leaves grew erect. At Naas Creek in the Snowy Mountains spreading plants *(Marsden 178B)* were found growing on the banks of the stream, whilst erect specimens *(Marsden 178A)* of apparently the same species were growing below permanent water level. When plants from each habitat were cultured together in the laboratory they were indistinguishable except for size, the plants from the banks being generally smaller. Small grass-like specimens of *I. muelleri* from rock pools in central and southern Australia also grew erect when submerged and rather spreading when grown in wet soil.

Despite this morphologic plasticity, at least some of the variation between populations appears to be genetically based. Plants from ephemeral shallow rock pools in central Australia (e.g. fig. le) and plants from ephemeral swamps in south-eastern Australia remained distinct from plants from permanent water in the Snowy Mountains and Tasmania, even when grown under the same conditions for two years. Those from less permanent water remain smaller, with fewer, more slender leaves. These plants grow from late autumn to spring and die off to a resting stage in the corm during summer. Those from permanent waters remain green all year round, shedding the old sporophylls as they are pushed off by new growth. Plants from the ephemeral conditions can be kept green all year round if submerged in permanent water, although they usually lose most of their leaves during the late summer and autumn.

Despite the differences between the extremes of form shown by plants included in *I. muelleri,* there is almost complete intergradation from one extreme to the other (fig. la-g).

Fig. 1 Variation in plant size and habit of *I. muelleri* from several localities, scale = 10 cm.a. *Marsden 177;* b. *Beauglehole 47901 B;* c. *Marsden 178B;* d. *Marsden 39;* e. *Beauglehole 45893;* f. *Beauglehole 36218;* g. *Marsden* 150.


G
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Lobing of the corm

The corm-like stems of *Isoetes* usually bear 2 or 3 (occasionally 4 or more) deep furrows along their length resulting in a lobing of the corm.

In the type description (Braun, 1868), *I. muelleri* was described as having a three lobed corm. However, in this study populations of *I. muelleri* have been found to contain from 5-50% bilobed plants. Taxonomic use of this character has thus led to considerable confusion in the classification of this species and bilobed specimens of *I. muelleri* have often been misidentified as *I. humilior*. Clute (1905) noted similar variation in lobing of some unspecified North American species. However, Pfeiffer (1922) considered the number of lobes of the corm to be characteristic for each species, with only a low frequency of deviation within species from the typical number of lobes.

Number of corm lobes was one of the key characteristics used by Braun (1868) to distinguish between *I. muelleri, I. stuartii* and *I. humilior,* but in view of the evidence for corm variation in *I. muelleri,* this feature is less distinctive than considered by Braun.

Stomata

Presence or absence of stomata on leaves of *Isoetes* is a character which has been traditionally correlated with habitat. Terrestrial and amphibious species always possess stomata whilst they are generally lacking in aquatic species, although there are some exceptions to this latter case (Pfeiffer, 1922). Consequently emergent and submerged plants of *I. muelleri* might be expected to possess and lack stomata respectively.

However, plants of *I. muelleri* have always been found to bear some stomata, at least on the apical portion of the leaves, even when growing permanently submerged. When emergent plants were transferred to aquatic conditions, the stomatal frequency was observed to diminish on the new leaves produced underwater.

The presence of stomata in *I. muelleri* and their absence in *I. stuartii* and *I. humilior* was another feature used by Braun (1868) to separate the species. However, plants recently collected from Tasmania (*Morris,* Elizabeth River) which otherwise corresponded to the description of *I. stuartii* were found to possess a few stomata on the apical portions of the leaves. Hence this feature also appears to be inconsistent and of doubtful taxonomic use for the separation of this species from *I. muelleri. I. humilior* appears consistently to lack stomata.

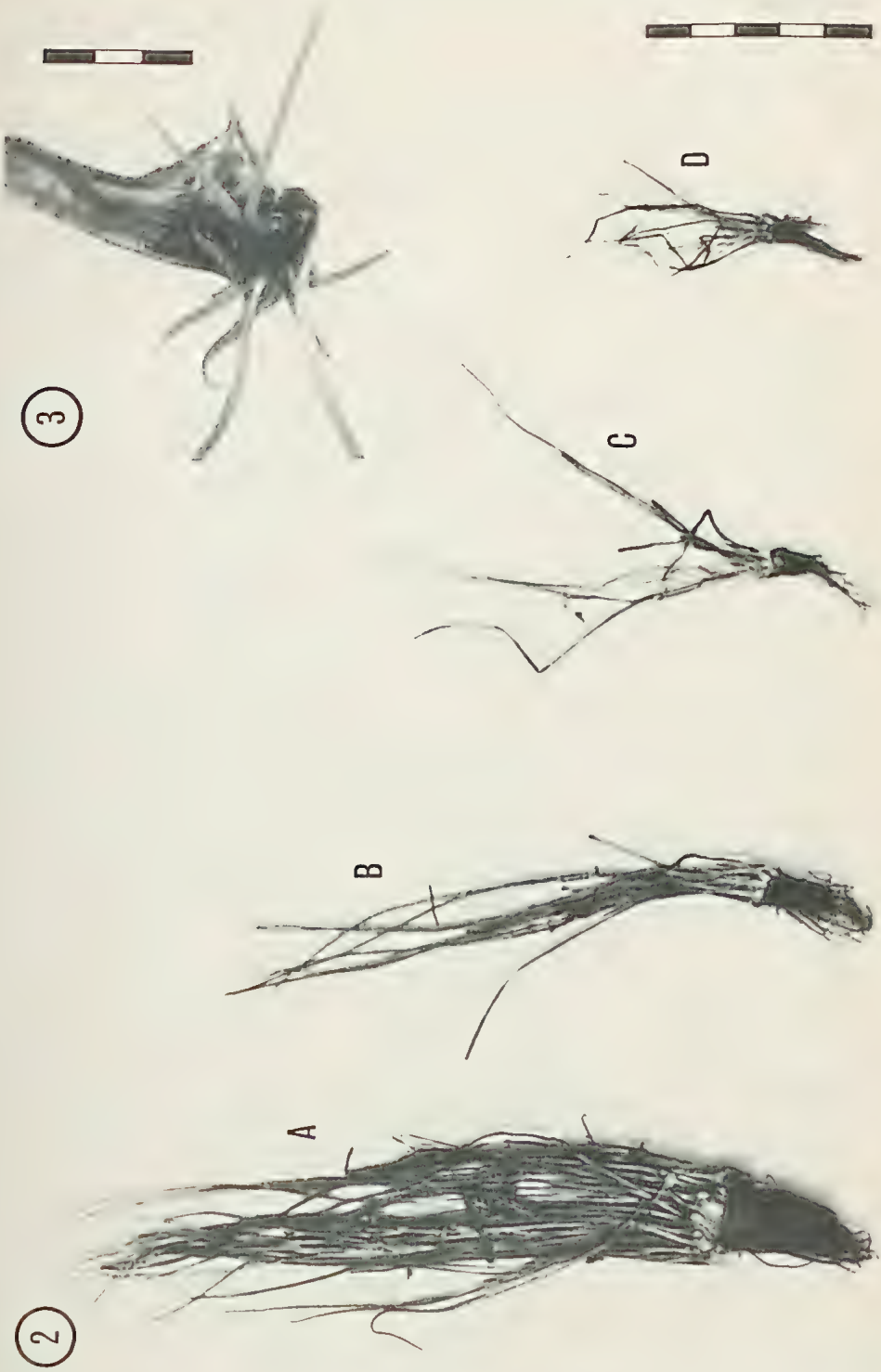
Megaspores

Megaspore ornamentation has been one of the most widely used taxonomic characters in *Isoetes.* The large size of these spores enables observation of gross ornamentation features using only relatively low magnifications such as are available with a hand lens. The advent of scanning electron microscopy has revolutionized examination of such spores, facilitating not only observations of gross ornamentation, but also study of the ultra-structure of the outer spore walls.

Polymorphism of megaspores within individual sporangia has been well documented for species from America (Jeffery, 1937), India (Pant and Srivastava, 1962; Goswami and Arya, 1970) and Africa (Hall, 1971). Goswami and Arya (1970) described the different spore forms as large, medium and small or, in the case of dimorphic spores, as large and small. Similar notation for megaspores was used by Hall (1971) and Marsden (1976). This terminology can lead to considerable confusion when discussing megaspores and can be misleading as the small megaspores of dimorphic types are analogous with the medium spores of a trimorphic type. Also the small megaspores of one species may be about equal in diameter to the large megaspores of another species which has normally diminutive spores.

Fig. 2. Range in plant size within a single population of *I. muelleri; Marsden 30,* scale = 5 cm.

Fig. 3. Young sporelings growing from within sporangium freshly removed from plant of *I. muelleri, Marsden 177,* scale = 3mm.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The need is thus indicated for the adoption of acceptable terminology to define megaspore type and to ensure clarity in description. The following grouping system is proposed:

Type I megaspores (fig. 4)

Almost spherical in shape, nucleate and containing large quantities of fats and oils and other storage products; usually fertile.

Type IIA megaspores (fig. 4)

Somewhat flattened and usually triangular in outline, enucleate and almost totally devoid of storage compounds; infertile.

Type IIB megaspores (fig. 4)

Flattened and triangular in outline, enucleate, and lacking any storage compounds; infertile. (So far, recorded for only two species, *I. pantii* Goswami and Arya, and *I. indica* Pant and Srivastava).

Type III megaspores (fig. 4)

Irregular, dumb-bell shaped megaspores, usually appearing like parts of two Type I megaspores fused or joined together by one or more tubular connections, probably binucleate, and containing other storage products; possibly fertile. (Occur only in very low frequencies in sporangia containing Type I and Type IIA megaspores).

Type I megaspores are larger than, and quite distinct in shape from Type IIA megaspores whilst in any one species these are larger in turn than Type IIB megaspores Approximate relative sizes of the different megaspore classes are shown in figure 4. The actual size of Type I and Type IIA megaspores, however, varies greatly between species, e.g. the Type IIA megaspores of *I. coromandelina* L.f. (Marsden, 1976) may be as large as some of the Type I megaspores of *I. muelleri.*

Type IIA and Type IIB megaspores differ mainly in size and nature of the spore wall layers (Goswami and Arya, 1970). The size range of these spore types may, however, overlap in different species, e.g. Type IIB megaspores of *I. indica* may reach 380μm in diameter (Goswami and Arya 1970) while Type IIA megaspores from other species may also be in this size range. When the contents of individual sporangia from *I. indica* or *I. pantii* were examined it was found that the Type IIB megaspores occur as a distinct size group. Because of the similarities between the two smaller megaspore size groups from these species, they are classified as subgroups of one type (Type II) of megaspores. In species with only one megaspore type, this corresponds to the Type I megaspore group of species with polymorphic megaspores. Possible origins of the different megaspore types are discussed later in this paper.

Pfeiffer (1922), in her monograph on *Isoetes*, divided the genus into four sections — *Tuberculatae* Pfeiffer, *Cristatae* Pfeiffer, nom. illegit.*, *Echinatae* Pfeiffer, *Reticulatae* Pfeiffer — on the basis of megaspore ornamentation. De Vol (1972) proposed a fifth section, *Psilatae* De Vol, for species with smooth megaspores, but this name has not been validated by a Latin description as required under Article 35 of ICBN. Pfeiffer appears to have referred only to those megaspores classified here as Type I megaspores in her discussion as the size ranges given for some species, now known to have dimorphic megaspores, do not include the size range of the Type II megaspores (e.g. *I. coromandelina* and *I. muelleri).*

The megaspores of *I. muelleri* were described by Braun (1868) as covered with numerous, low, uneven tubercles, some of which were fused together into confluent ridges. Thus this species was placed by Pfeiffer (1922) into the section *Tuberculatae.* Examination of a wide range of specimens has revealed that *I. muelleri* megaspores are always dimorphic in size, with Type I and Type IIA megaspores, in approximately equal numbers, and occasional Type III megaspores occurring within individual sporangia.

*Pfeiffer's sectional name *Cristatae* is illegitimate as it contains the type species of the genus and following Article 22 ICBN must be named sect. *Isoetes.*

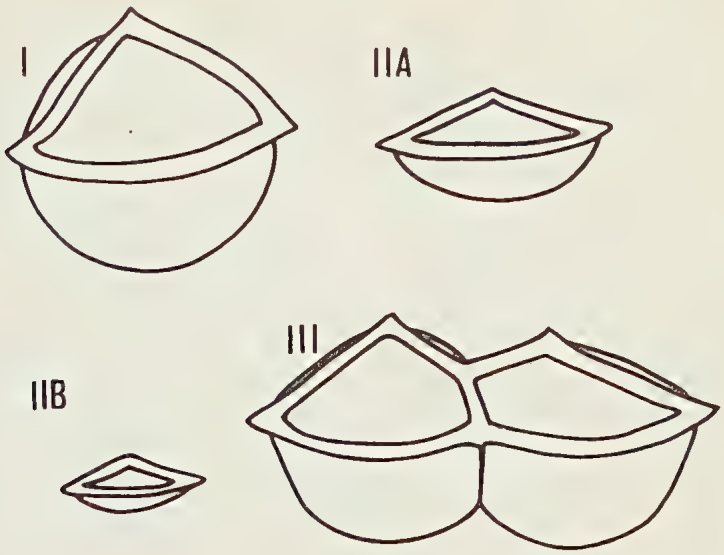


Fig. 4. Diagramatical representation of the relative sizes and shapes of Type I, Type IIA, Type IIB and Type III megaspores.

Type I megaspores, the only megaspore type previously described and discussed for this species, were found to be only very rarely tuberculate, with many specimens, including those from the nomenclatural type (fig. 11) showing only few tubercles, mainly on the proximal faces, and ridges of varying size and confluence (figs. 5, 7, 9, 11). Commissural and triradiate ridges of the spores are usually quite prominent, with the triradiate ridge usually somewhat broader and less raised than the commissural ridge. The range of Type I megaspores further includes spores showing irregularly confluent ridges only (fig. 13) through to others covered by a definite reticulate pattern (fig. 15). On the basis of this character alone, *I. muelleri* could be placed in any one of three different sections of the genus.

Similar infraspecific variation was recorded by Duthie (1929) for African species, and cases such as these cast serious doubt on the usefulness of this classification system for subdivision of *Isoetes*.

Type IIA megaspore patterning shows even wider variation than that of the Type I megaspores of *I. muelleri* (figs. 17-21) varying from almost smooth (except for the triradiate and commissural ridges) to closely reticulate.

Often there are wide differences between Type IIA megaspores from within individual sporangia of *I. muelleri* (figs. 19, 20).

Ornamentation of Type III megaspores (fig. 22) usually closely resembles that of Type I megaspores for that species.

Perispore of megaspores

The possible taxonomic usefulness of perispore structure of *Isoetes* megaspores examined using scanning electron microscopy was first demonstrated by Wanntorp (1970). Wanntorp found differences in perispore structure between species of *Isoetes* from south-west Africa, while Taylor et al (1975) found it was possible to separate two closely related species from North America on the basis of perispore structure.

The siliceous perispore of *I. muelleri* Type I megaspores is most commonly covered with minute, twisted spines (fig. 14) usually present in very great numbers (fig. 12, 16). In plants of a few collections, and in specimens corresponding to Braun's description of *I. stuartii,* these spines are poorly developed (fig. 8, 10), or scarcely present (fig. 6), however, an almost continuous range of variation between the two extremes has been found (see sequence figs. 6, 8, 10, 12, 14, 16).

In order to ensure that the observed variation is not simply due to megaspore age differences, a range of megaspores of different ages has been examined.

Normally when megaspores for scanning electron microscopy were chosen, they were initially examined using a light microscope, prior to preparation, and only mature spores were used. Immature spores either collapse when dried or have a pale translucent appearance when viewed in transmitted light. Therefore, a range of megaspores, from the youngest which did not collapse when dried but which were still obviously immature, to the oldest megaspores present on the plant, were examined from both plants which normally show few spines on the Type I megaspores as well as plants which have dense spinulose megaspore perispore surfaces. Results of this study are shown in figures 23-28.

Immature spores from plants with Type I megaspores showing poorly developed spines *(C. Marsden 177)* were examined and found to have an amorphous outer coating, which was shown by secondary X-ray analysis to contain considerable silica. Type I megaspores from sporophylls only two positions sequentially further out from the immature sporangia, were also examined. Although these megaspores were only slightly older they were found to have a perispore structure (fig. 25) almost identical with that of the oldest Type I megaspores on the plant.

Similar results were observed for specimens *(Beauglehole 52587)* which normally have spiny perispore surfaces. Figure 26 shows an immature spore with the early stages of formation of the small spines (fig. 27) visible in the perispore structure. The next oldest sporangium on the plant contained Type I megaspores with almost completely developed spines already present (fig. 28).

Since numerous leaves are produced and shed each season, age differences between sequential sporangia formed would be expected to be, at the most, only a few weeks. Thus the perispore patterning is apparently laid down very rapidly, after which almost no further perispore development takes place.

Perispore patterning of Type IIA and Type III megaspores of *I. muelleri* closely resembles that of the corresponding Type I megaspore in each individual plant.

Megaspore size

Megaspore size is a character used extensively by Pfeiffer (1922) in her key to species. Again, only those megaspores equivalent to Type I megaspores were considered by her.

Variation in size ranges of diameters of both Type I and Type IIA megaspores from several populations of *I. muelleri* are shown in figures 29, with the arithmetic mean, standard deviation from the mean and absolute size ranges indicated.

Figs. 5-10. Scanning electron micrographs of Type I megaspores of *I. muelleri.*

Fig. 5. Distal face of megaspore, *Marsden 178A*, scale = 200 μm.

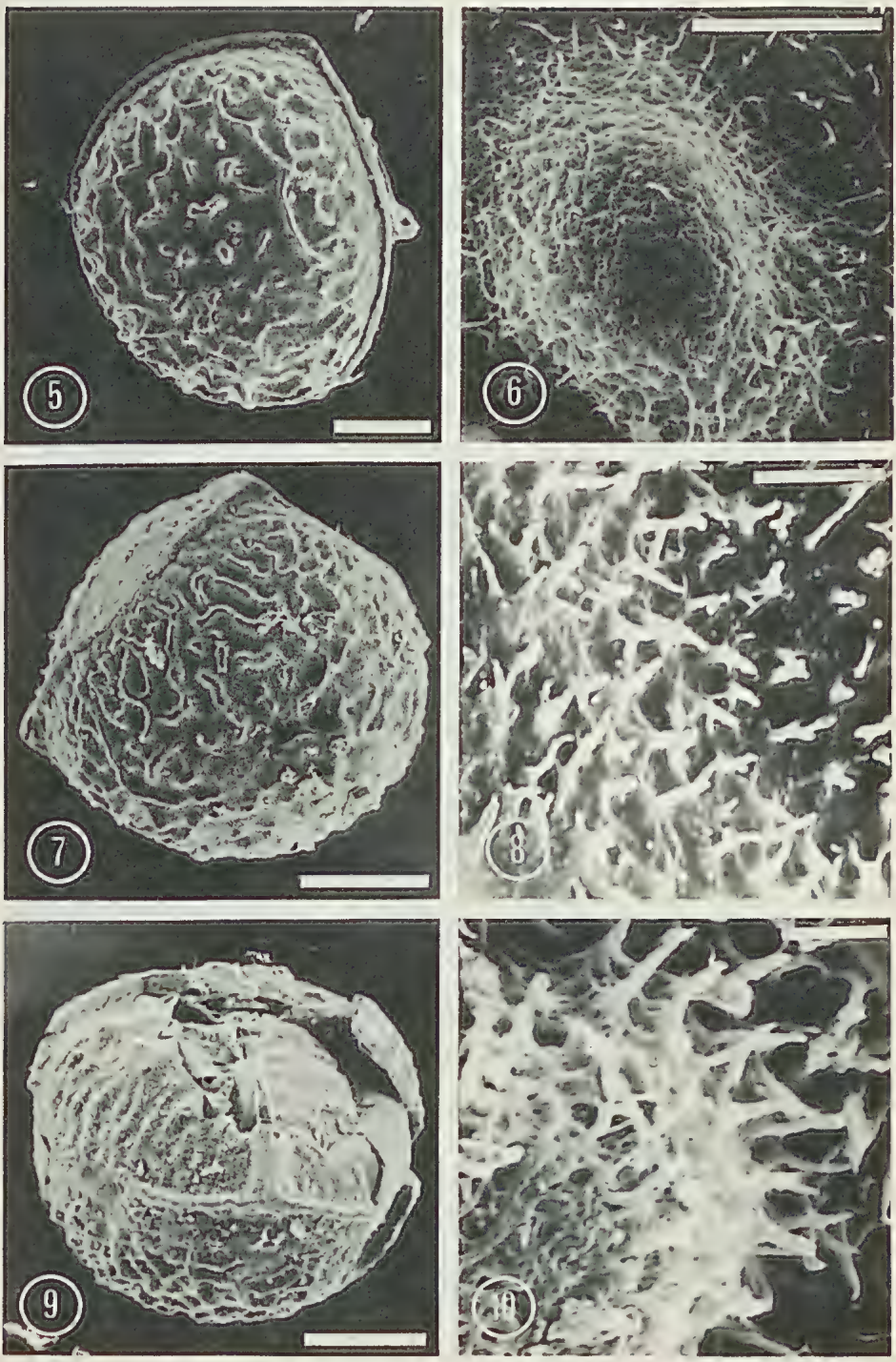
Fig. 6. Detail of surface of megaspore in fig. 5, scale = 20 μm.

Fig. 7. Distal face of megaspore, *Marsden 133*, scale = 200 μm.

Fig. 8. Detail of surface of megaspore in fig. 7, scale = 5 μm.

Fig. 9. Side view of megaspore, holotype *I. stuartii*, scale = 200 μm.

Fig. 10. Detail of surface of megaspore in fig. 9, scale = 5 μm.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Type I megaspores were found to vary from 560-750 μm *(Marsden 177)* down to 360-440 μm *(Seppelt,* Tassie Creek) in diameter whilst Type IIA megaspores varied from 380-520 μm *(Marsden 133)* to 250-320 μm *(Seppelt,* Tassie Creek) in diameter. Although the size range for Type I megaspores from one locality may fall within that of Type IIA megaspores from another, the differences in shape and contents are sufficient to distinguish between these spore types.

Megaspores of *I. muelleri* are much more variable in size than in any other species of *Isoetes* so far studied, e.g. megaspores of the two subspecies of *I. coromandelina,* are relatively similar, despite the occurrence of one subspecies in India and the other in Australia (Marsden, 1976). However, the continuous variation in size is indicated by the plot of size data in figure 29, with no discontinuities apparent.

Microspores

Formation of microspores by *I. muelleri* is very rare. In the range of material examined during this study only one small specimen, a plant grown in culture, from the south-east of South Australia *(Marsden 11)* was found to produce microspores. Prior to being placed in culture the plant appeared to have produced only megaspores, but no megasporangia were evident once production of microsporangia had begun. Unfortunately no mature microspores were obtained from this plant as it was fixed for examination of meiosis at an early stage of growth.

Velum

Braun (1868) described the velum of *I. muelleri* as complete and closed. However, whilst complete or almost complete coverage of each sporangium by a velum has been found most commonly, some specimens with only a half to a third of each sporangium covered have also been found.

In specimens with an incomplete velum, the extent of coverage of the sporangia was occasionally found to vary considerably on each plant, most often with narrower vela on the outer sporangia. Similar variation was also noted for a few specimens which corresponded to the description given for *I. stuartii,* which Braun (1868) described as having complete and closed vela. Plants identified as *I. humilior* were also found to have a complete velum in all specimens examined.

Sporangia

Sporangia in *I. muelleri* vary greatly in size from small (2 x 2 mm) in some of the smallest plants, to moderate sized (9 x 5 mm) in very large plants. Small sporangia contain only about 20-30 megaspores whilst the larger ones may contain as many as 200 or more.

The sporangia vary in shape from orbicular in the smaller sporangia to elliptic or obovate in the largest. The sporangial shape does not vary as greatly within individual specimens of the two sub-species of *I. coromandelina* (Marsden 1976).

Cells of the sporangial wall of *I. muelleri* are only infrequently pigmented as described by Braun (1868). This pigmentation was another feature used by Braun to differentiate *I. muelleri* from *I. stuartii* in which there is little pigmentation.

Figs. 11-16 Scanning electron micrographs of Type I megaspores of *I. muelleri.*

Fig. 11. Side view of megaspore, lectotype *I. muelleri,* scale = 200 μm.

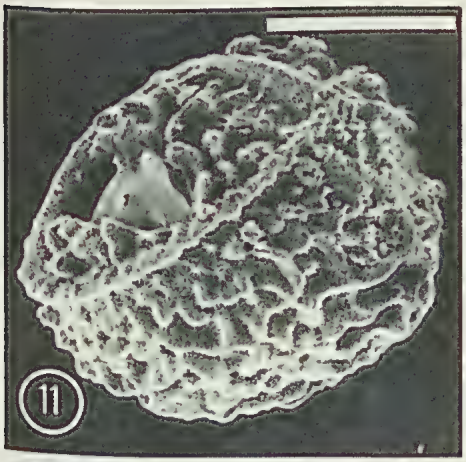
Fig. 12. Detail of surface of megaspore in fig. 11, scale = 5 μm.

Fig. 13. Distal face of megaspore, *Marsden 35,* scale = 200 μm.

Fig. 14. Detail of surface of megaspore in fig. 13, scale = 5 μm.

Fig. 15. Distal face of megaspore, *Beauglehole 44864,* scale = 200 μm.

Fig. 16. Detail of surface of megaspore in fig. 15, scale = 20 μm.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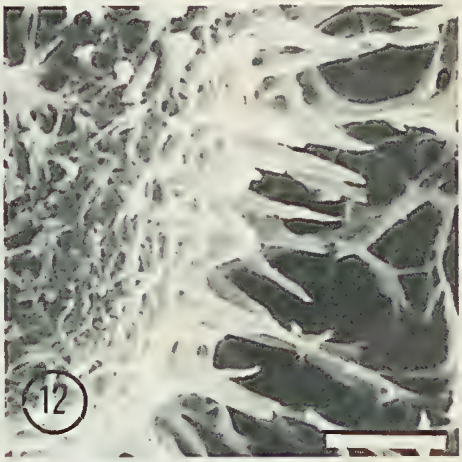
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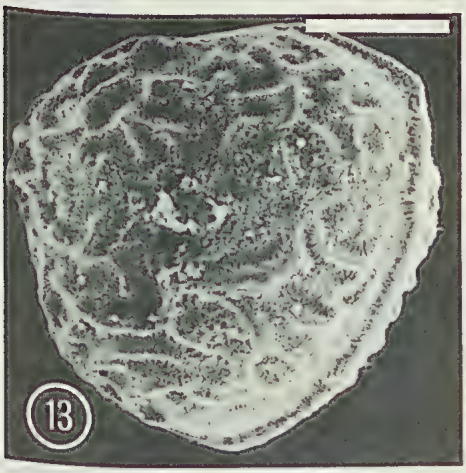
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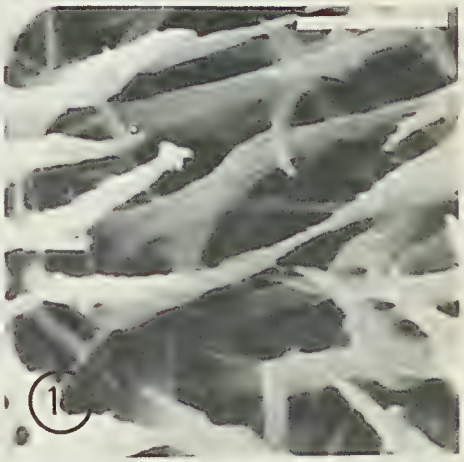
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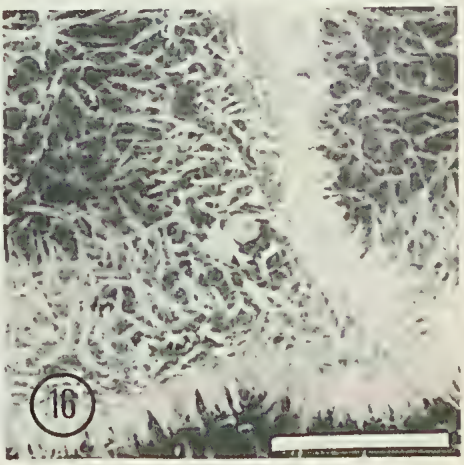
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Cytology

Chromosome numbers published for *Isoetes* species have shown a remarkably constant base number of n = 11 with polyploids occurring in several species (Abraham and Ninan, 1958; Jermy, 1964; Pant and Srivastava, 1965; Matthews and Murdy, 1969; De Vol, 1972; Rychlewski and Jankun, 1972).

Chromosome counts have been made for several populations of *I. muelleri* and a partial polyploid series has been found. Diploid (2n = 22) *(Marsden 4),* tetraploid (4n = 44) *(Marsden 177, 178A, 178B; Wollaston,* Marcollate Rocks; *Symons,* Carrappee Hill) and pentaploid (5n = 55) *Marsden 11, 31, 32, 39 Beaglehole 45893)* populations of *I. muelleri* have been noted, this being the first known record of pentaploids in the genus.

All chromosome counts have been based on observations of mitotic divisions. Meiosis has been observed only once in *I. muelleri* from a single pentaploid specimen which had been cultured in the laboratory *(Marsden 11).* At metaphase univalents, bivalents, and multivalents were clearly visible.

Formation of Type I, Type IIA and Type III megaspores in all plants of *I. muelleri,* even in diploids, indicates that meiosis probably follows an irregular pattern such as that elucidated for *I. coromandelina* from India (Verma, 1960; 1961; Pant and Srivastava, 1965). This irregular meiosis leads to the production of chromosomally unreduced Type I megaspores and enucleate Type IIA megaspores from a mitotic-like division followed by a second cytokinesis (Verma, 1960; 1961). The origin of Type III megaspores has been discussed by Jeffery (1937) who considered that these dumb-bell shaped spores were the result of an abortive second division of meiosis. These spores would be binucleate. Pant and Srivastava (1965) described a possible origin for Type III megaspores which would result in one part being nucleate and the other part enucleate, i.e. much like a Type I and a Type II megaspore fused together. The exact nature of these spores in *I. muelleri* is not understood as only a limited amount of live material has been available, and cells undergoing meiosis have been difficult to find. If the large dumb-bell spores are binucleate, and in rare instances underwent fusion of these nuclei, germination of these spores could be a possible source of polyploids.

Type I, Type IIA and Type III megaspore production in diploid, as well as in polyploid plants of *I. muelleri* indicates that some mechanism besides polyploidy is inducing irregular meiosis and irregular spore production.

Apomictic germination of diploid Type I megaspores has been described by Jeffery (1937) and Pant and Srivastava (1965) for other species of *Isoetes.* Similar growth of Type I megaspores occurs in *I. muelleri* with numerous sporelings from the previous year's megaspores, often appearing in the soil around the base of mature plants at the start of each growth season, apparently with total lack of microspores. Growth of these sporelings could explain the origin of the very dense colonies of *I. muelleri* sometimes found in rock pools (e.g. on the summit of Ayers Rock in Central Australia).

Occasionally Type I megaspores may commence growth whilst enclosed within sporangia which are still attached to living plants (fig. 3). This is probably similar to the apomixis recorded by Sadebeck (1902) for *I. lacustris* L. and *I. echinospora* Dur. Germination of such spores in *I. muelleri* has only been noted in aquatic specimens, and it is noteworthy that both *I. lacustris* and *I. echinospora* are also aquatic species.

Figs. 17-22 Scanning electron micrographs of Type IIA and Type III megaspores of *I. muelleri.*

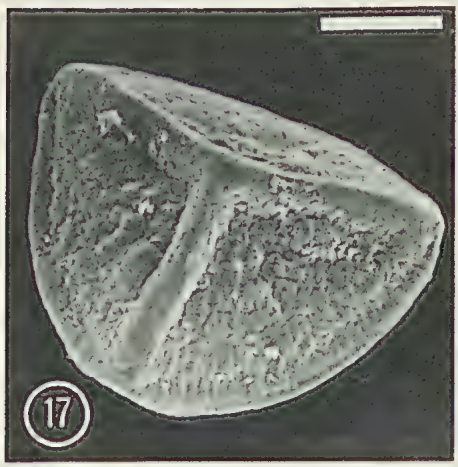
Fig. 17. Proximal faces of Type IIA megaspore, *Marsden 178A,* scale = 150 μm.

Fig. 18. Distal face of Type IIA megaspore, *I. muelleri* lectotype, scale = 150 μm.

Figs. 19, 20. Side views of type IIA megaspores, *Marsden 35,* scale = 150 μm.

Fig. 21. Proximal faces of Type IIA megaspores, *Beauglehole 44864,* scale = 150 μm.

Fig. 22. Proximal faces of Type III megaspores, *Marsden 178A,* scale = 300 μm.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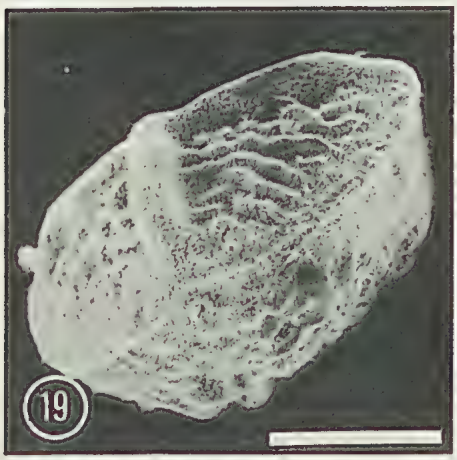
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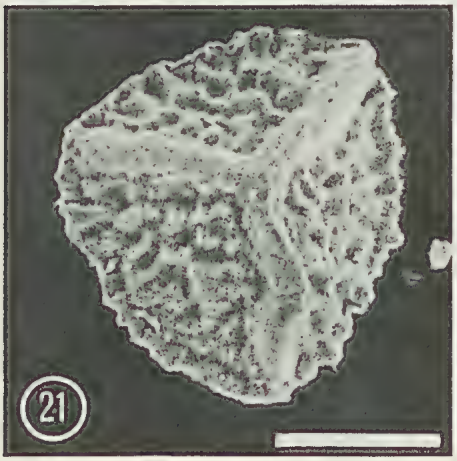
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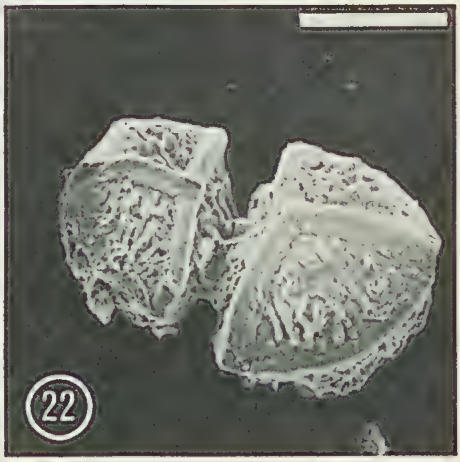
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The occurrence of polyploidy and apomixis in *I. muelleri* may largely explain the variation observed in this species, and the wide range of habitats colonized including cold sub-alpine waters, temperate, seasonal swamps in southern Australia, and ephemeral rock pools on granite outcrops in arid regions of central Australia.

Conclusions

Throughout the range of characters studied, *I. muelleri* shows very wide variation. However, no distinct infraspecific groups are apparent. Each character examined shows a more or less continuous range of variation which for many features exceeds the limits normally associated with individual species of *Isoetes*.

At one extreme of form of *I. muelleri* are large aquatic plants (fig. la) which have large sporangia and the largest megaspores, the perispore of which bear only a few spines (fig. 5, 6). At the other extreme are rather small, amphibious plants which have small to medium sized sporangia, contain small, or moderately sized megaspores reticulately ornamented (fig. 15) and densely covered with minute spines (fig. 16). The type specimen of *I. muelleri* fits between these two extremes.

I. humilior, is quite distinct from *I. muelleri*, having thick, rigid, dark leaves quite unlike any from the range of *I. muelleri;* corms of *I. humilior* have two rather elongated lobes whilst those of *I. muelleri* are compact and short; *I. humilior* produces only Type I megaspores, which are almost smooth, and also produces microspores; the leaf bases of *I. humilior* are thick and rigid, whilst those of *I. muelleri* are membranous and quite flexible. Thus, *I. humilior* on the basis of these features is retained as a distinct species.

I. stuartii, described by Braun (1868) in the same paper as *I. muelleri*, was distinguished on the basis of habitat, occurrence of stomata. lobing of the corm and colouring of the sporangial walls. All of these characters have been found to vary in *I. muelleri* as well as other features such as plant size and habit, sporangial characteristics and megaspore size and ornamentation. Thus *I. stuartii* is now considered to be conspecific with *I. muelleri*. The type of *I. muelleri* is more representative of the species than is that of *I. stuartii*. *I.stuartii* has also frequently been confused with *I. humilior* (Pfeiffer, 1922). Therefore, it is here proposed that *I. stuartii* be reduced to synonomy under *I. muelleri*.

I. muelleri A. Braun, Monatsber. K. Akad. Wiss. Berlin, 541, 1868; Pfeiffer, Ann. Mo. Bot. Gdn, *9*, 126, 1922.

Lectotype: Queensland, wet places near Rockhampton, *P. O'Shanesy*, 1867 (B!), (Syntype in K!)

Syn. *I. stuartii* A. Braun, Monatsber. K. Akad. Wiss. Berlin, 539, 1868.

Holotype: Tasmania. South Esk River, *C. Stuart*. (MEL!)

Figs. 23-28. Scanning electron micrographs of developing Type I megaspores of *I. muelleri*.

Fig. 23. Immature Type I megaspore distal-face, *Marsden 178A*, scale = 100 μm.

Fig. 24. Detail of surface of spore in fig. 23 showing amorphous siliceous perispore, scale = 10 μm.

Fig. 25. Detail of surface of slightly older megaspore from same plant as figs. 23, 24, showing fully developed surface structure as in fig. 6, scale = 10 μm.

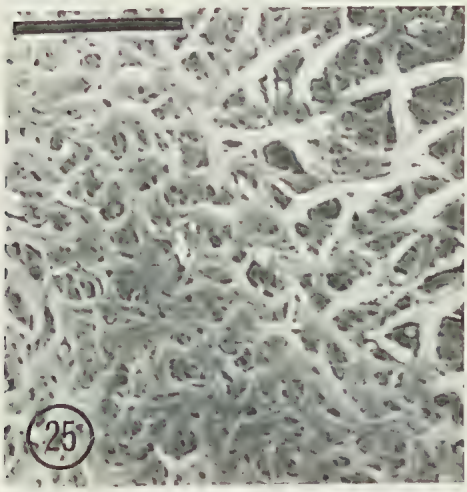
Fig. 26. Immature Type I megaspore, proximal faces, *Beauglehole 47901*, scale = 200 μm.

Fig. 27. Detail of surface of spore in fig. 26 showing beginnings of development of spines on surface of perispore, scale = 10 μm.

Fig. 28. Detail of surface of megaspore from next oldest sporangium than that in fig. 26 showing well developed spines on perispore surfaces, scale = 10 μm.


23


24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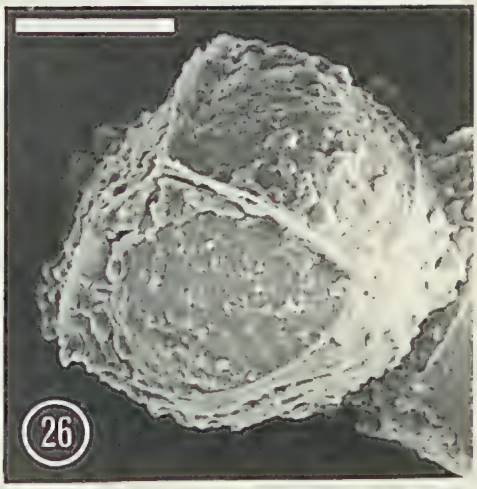
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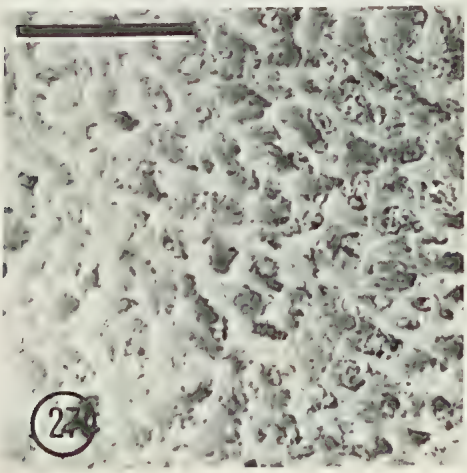
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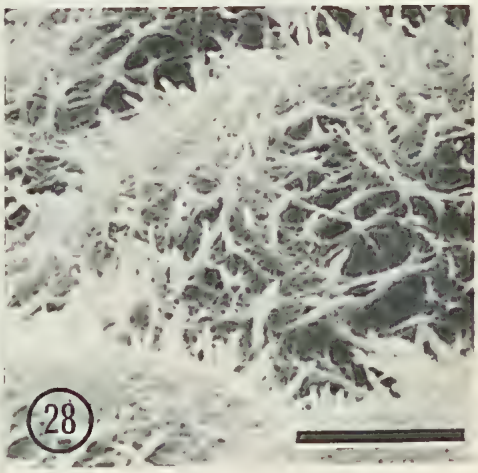
28

Diagnosis

I. muelleri is distinguishable from other Australian species by the presence of sporangial vela and occurrence of imorphic spores. In Australasian species of *Isoetes*, dimorphic spores are known only from *I. coromandelina* L. f. ssp. *macrotuberculata* C. Marsden (Marsden, 1976) and *I. muelleri* but *I. coromandelina* lacks vela covering the sporangia.

Distribution

I. muelleri is the most widespread species of *Isoetes* in Australia occurring in all states and territories. A map showing the known distribution is given (Map 1).

Representative collections examined

Details are only included for collections referred to in the text.

SOUTH AUSTRALIA: S.E. of S.A. 1 km E. of Comaum Forest, 15.vi. 1973, *Marsden II* (AD); 19.xii. 1973, *Marsden 32* (AD); S. edge of Comaum Forest, 18.xii. 1973, *Marsden 30* (AD); W. edge Comaum Forest, 19.xii. 1973. *Marsden 35* (AD); S.E. of S.A., Wrattonbullie station, 19.xii. 1973, *Marsden 39* (AD); S.E. of S.A., Marcollat Rocks, 19.x. 1974, *E. M. Wollaston* (AD); Eyre Peninsula, Tassie Ck., 23.viii. 1973, *R. D. Seppelt* (AD); Eyre Peninsula, Carrappee Hill, 12.ix. 1974, *D. E. Symon 9052* (AD).

NORTHERN TERRITORY: Palm Valley, 25.vi. 1974, *A. C. Beauglehole 45893* (MEL); MacDonnell Range, Trephina Gorge, 1.vi. 1974, *A. C. Beauglehole 44864* (MEL).

NEW SOUTH WALES: (incl. A.C.T.): Snowy Mountains, 1.7 km W. Kiandra, 19.i. 1975, *Marsden 177* (AD); Snowy Mountains, Naas Creek, 25.i. 1975 *Marsden 178A, 178B* (AD).

VICTORIA: East Gippsland, Forlorn Hope Plain, 19.i. 1971, *A. C. Beauglehole 36218* (MEL).

TASMANIA: Shannon Lagoon, 30.xi. 1974, *Marsden 133* (AD); 2.xii. 1974, *Marsden 150* (AD); Elizabeth River at Campbelltown, 1973, *D. Morris* (ADU).

WESTERN AUSTRALIA: Mt Madden, 9.viii. 1975, *Marsden 205* (AD); Kimberley's, Galvin's Gorge, 24.vii. 1974, *A. C. Beauglehole 47901* (MEL).

Acknowledgements

I wish to thank Dr E. M. Wollaston for her encouragement and helpful comments on the manuscript. I am also grateful to Dr J. P. Jessop and Mr R. J. Chinnock for their helpful suggestions, and to the Directors of the South Australian State Herbarium (AD); Botanic Garden and Botanic Museum, Berlin (B); Herbarium Australiense (CANB); the Herbarium, Royal Botanic Gardens, Kew (K); the National Herbarium of Victoria (MEL) and the Herbarium of the Northern Territory (NT) for loan of specimens.

I also wish to thank Mr D. Morris, Mr A. C. Beauglehole and Mrs A. C. Alcock for sending numerous specimens.

This study was assisted by a grant under the Australian Biological Resources Study.

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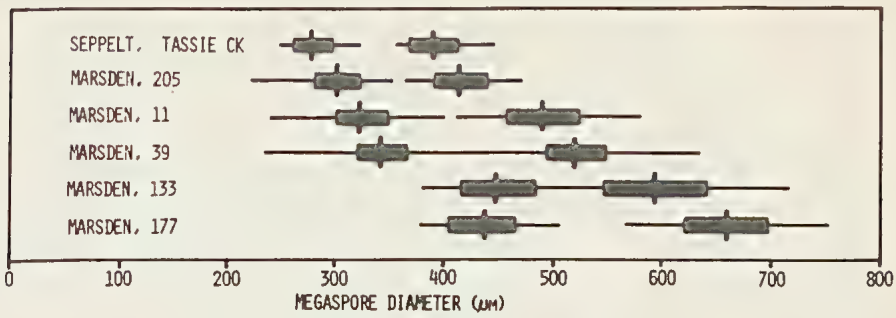
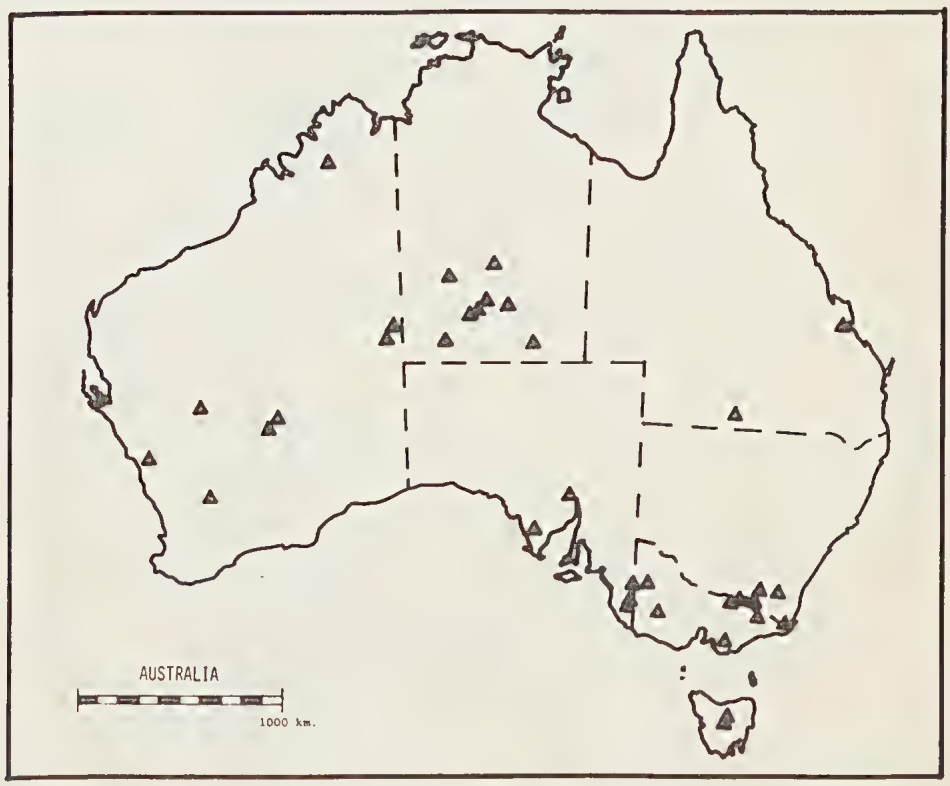


Fig. 29. Plot of megaspore diameters for six populations of *I. muelleri* showing the arithmetic mean, standard deviation (broad bands) and size range (narrow bands).



Map 1. Distribution map of *I. muelleri*.

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